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THE EFFECT OF CAUTERIZING THE PPM ("GOLD SPOTS" OF AUTHORS) OF THE PUPA OF THE MONARCH BUTTERFLY (*D. plexippus*)

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INTRODUCTION

It is the purpose of this paper to describe the effect of microcauterizing the Prismatic Pigmented Maculae (PPM), commonly referred to as "gold spots," of the monarch butterfly (Fig. 1). We have chosen the terminology "prismatic pigmented maculae" in place of "gold spots" for the following reasons:

The word "gold," as a descriptive term, is meaningless, nor would the use of the word "golden" be much of an improvement. The choice of the word came about because of the yellow metallic luster associated with these particular areas (Fig. 1). In other species of Lepidoptera, however, such pigmented areas may be green, light blue, or a combination of colors to which the word gold would then not apply. Since metallic luster is a prismatic effect and since the areas are pigmented, we suggest the terms "prismatic pigmented" as being more descriptive and in keeping with the results of histological studies that have been published, as well as those carried out in our laboratory (unpublished).

The word "spot" is an ambiguous term. For example: "cold spot" refers to any one of the temperature spots where cold is normally perceived and thus refers to an area of sensitivity; it may refer to an area instantaneously affected by the impact of an electron beam in a cathode ray tube, thus referring to a light spot; and so on. In an attempt to avoid such ambiguity we suggest the use of the word "macula(ae)." This term is used extensively in biology, and more particularly in certain branches of medicine, as a "general term to designate an area distinguishable by color or otherwise from the surrounding area" — for

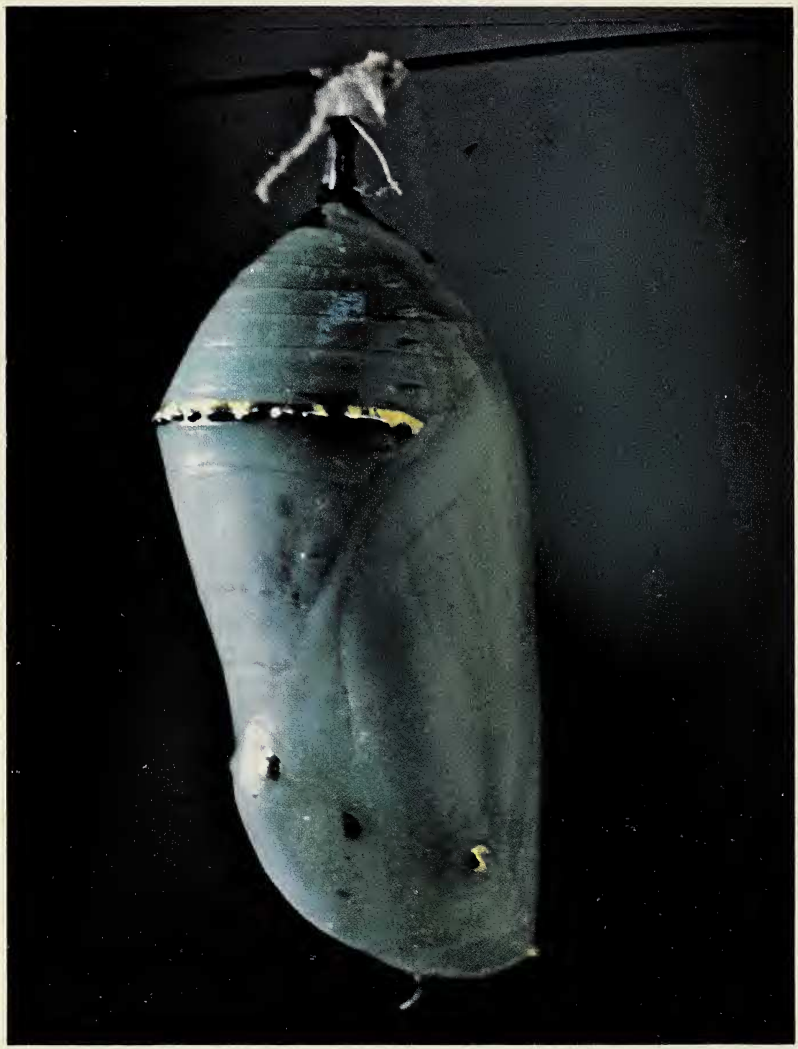

Fig. 1. Pupa of the monarch butterfly showing the prismatic pigmented maculae on one side.

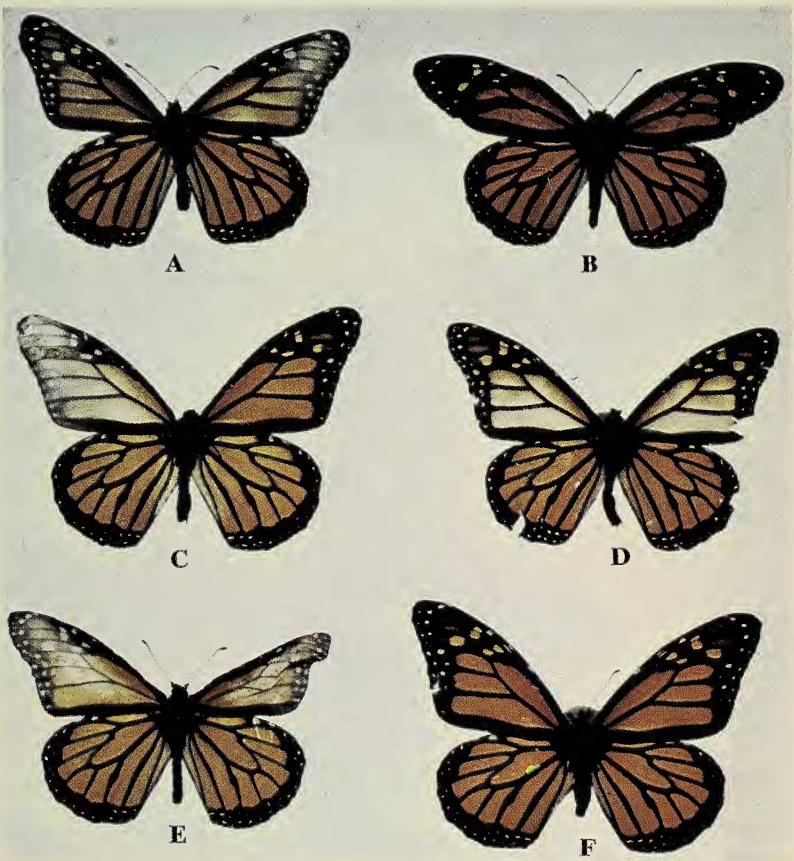

Fig. 2. Results of cauterization of the pupal LUPPMs and LNPPMs. A: Both LUs cauterized showing fading in both wing tips. B: Forewings distorted but no fading when area remote from PPMs is cauterized. C: Left LU and LN cauterized on left side only and showing extended area of fading on left forewing. D: Both LNs cauterized showing fading in central area of forewing and absence of apical fading. E: Cauterizing of LUs for 20 secs. showing extreme fading in left forewing and fading and distortion in right forewing. F: LUs cauterized for 3 secs. with no resultant fading (this specimen is taken as normal coloration for comparison with the other treated specimens in this plate).

example: "maculae caeruleae" referring to a purple area; or "maculae luteae" referring to a yellow area; and so on. We decided on such terminology because we are of the opinion that further research will indicate much more important functions for these structures than presented in this paper and a uniformity of terminology will permit a much clearer definition of the results obtained for discussion among those carrying out research in this area.

Thus, the structures found on the pupa of the monarch butterfly, considered in the present discourse, are referred to as *prismatic pigmented maculae,* which may be conveniently shortened to PPM. It is noted that some of the areas may not be prismatic, as in the case of the *ventral frontal* and *dorsal frontal* PPMs of the monarch butterfly pupae and which are also found in other species of Lepidoptera; these may be referred to as being *pigmented maculae.* If both the prismatic effect and color are absent then such areas may be designated as *"unpigmented maculae."* This permits a wider use of the terms while at the same time confining the description and the research in this field to a more logical presentation.

In order that such pupal structures may be discussed, Urquhart (1960) suggested terms for the various PPMs located on the pupa of the monarch butterfly (Fig. 3). The terms, for the most part, refer to the structures of the developing imago upon which the maculae are located. Thus "ocular" refers to the PPMs associated with the compound eyes; "lateral ulnar" to those located at the bases of the first pair of wings as distinct from those located on the wing ("alar"). The choice of the term "ulnar," which is perhaps not an applicable one, is used in the connotation of the ulnar joint of a vertebrate limb. "Lateral notal" refers to those located near the outside margin of the mesonotum; and so on. For convenience in textural reference these terms are shortened to "LU" for lateral ulnar; "LN" for lateral notal; and so on.

Urquhart (1960) suggested that the PPMs possibly acted as light receptors delaying emergence during periods of inclement weather. Petersen (1964) covered the spots with "fingernail polish" and concluded that either the polish was not effective in keeping out light rays or the PPMs do not function as photoreceptors during the emergence period. Although Petersen's experiment is by no means conclusive, it agrees with our studies (unpublished) in which the spots were covered with an opaque black enamel.

Taylor (1964) cut out the PPMs in some specimens and painted others with a black substance (material not stated). He found that all adults emerged from the pupae at the expected time and that the adults were normal.

PROCEDURE

The method of microcauterization described by Urquhart and Dampney (1964), in which a spark (fulguration) of a definite voltage was employed to destroy the tissues of the maculae, was used. The fulgurating point of the wire conductor was brought to within approximately 5 mm. of the macula to be treated. The time of fulguration was noted by use of a stop clock. Voltage was kept constant throughout all tests (2000 v.). The time of exposure varied: Some specimens were treated for one second in our initial trials; others for five seconds; ten seconds; and twenty seconds. This series of tests was carried out in order to ascertain the degree of damage to the cells of the maculae and the surrounding tissue.

As a control, specimens were cauterized in areas remote from the maculae.

In all, a total of 200 pupae were treated and of these 174 imagoes emerged. Lethality (26 specimens) was probably due to a viral infection and not the result of the cauterization (Urquhart, 1966).

Specimens were treated approximately one day after pupa ecdysis, a time period which, from our unpublished investigations, indicated that the cellular structure of the maculae was still unique and that the pigment was still present in the cells. It was, however, not possible to ascertain the exact hourly age of the pupa since eclosion at times took place early in the morning, or in the late evening, when the technician was absent from the laboratory. However, all pupae treated were in the range of 16 - 28 hours.

Thus, parameters such as pupal age, fulguration force (voltage), and time of exposure were kept as nearly constant as possible. However, the distance between the fulgurating point and the PPM was estimated under the dissecting microscope with the result that an error in judgment could have been made thus causing a difference in the degree of tissue destruction.

RESULTS

Specimens which were exposed for periods of 1 - 3 seconds indicated no wing fading (Fig. 2 F - this specimen is taken as normal pigmentation for comparison with the other specimens).

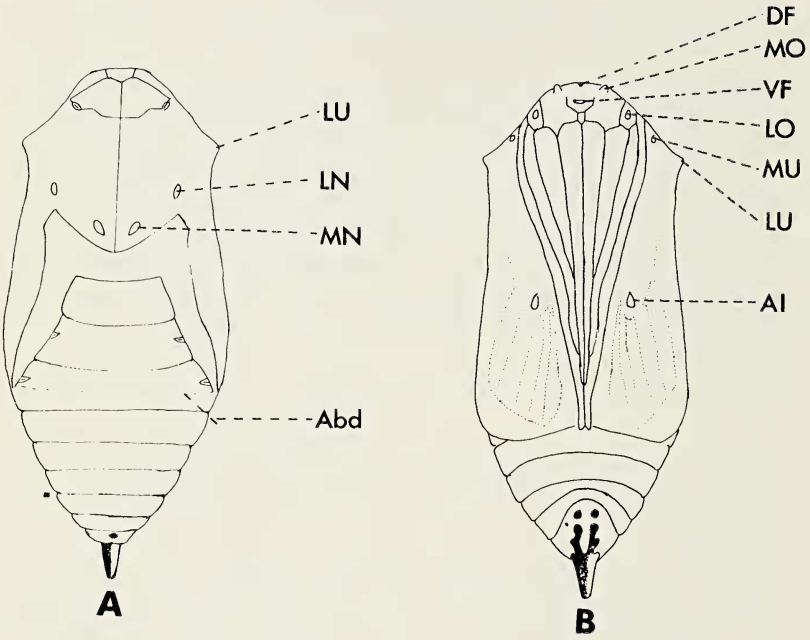

Fig. 3. Dorsal (A) and ventral (B) aspect of the pupa showing positions of the PPMs (LU: lateral ulnar; LN: lateral notal; MN: median notal; Abd: abdominal; DF: dorsal frontal; MO: median ocular; VF: ventral frontal; LO: lateral ocular; MU: median ulnar; Al: alar). VF and DF are non-prismatic and ephemeral — not discernible after 48 hrs.

Specimens exposed for a period of 5 - 8 seconds indicated fading in only a few of the specimens (20%). At ten seconds the majority of specimens indicated fading. At twenty seconds, although fading was evident in all specimens, the wings were, in many cases, distorted (Fig. 2 E). In all cases, fading of the scales occurred on both ventral and dorsal surfaces of the wing.

Of a total of 88 specimens in which both LUs were cauterized, 64 exhibited wing-tip fading (Fig. 2 A).

In some cases, when cauterization was extended beyond 10 seconds, or where the fulgurating point was too close to the PPM, fading tended to extend beyond the apical region (Fig. 2 E).

In order to test whether or not each of the pair of PPMs operated independently for each wing with which it was associated, only one of a pair of PPMs was treated and it was found that when the left PPM was cauterized only the left wing-tip faded and when the right one was cauterized only the right wing-tip indicated fading.

Of the controls (25 specimens) in which an area remote from the PPMs was cauterized for the same voltage and time, no fading was evident but the forewings were distorted (Fig. 2 B). The point of cauterization is seen at the branch of vein CU - M.

Of the remaining PPMs found on the body of the pupa the LNs and MOs were cauterized.

Of the 25 specimens in which the LNs were cauterized 18 indicated fading in the central portion of the wing and not at the wing-apex (Fig. 2 D).

When both LU and LN were cauterized on one side only the forewing of that side indicated fading for almost the complete wing area (Fig. 2 C).

When the area of the wing that had become faded was examined under the microscope it was found that the scales were, in some cases, normal in number per scale row but lacking pigment; others lacked pigment and there was also a reduction in the number per scale row; while still others were smaller in size and decidedly more elongate. In any one faded area one or more of these scale changes was found.

When the MOs were cauterized (25 specimens) all indicated a marked reduction in the number, or complete absence, of scales in the region of the fronto-clypeus and a distortion of the compound eyes due to the destruction of tissue beyond the area of the PPM (Fig. 4). In the latter case, the eyes tended to

Fig. 4. Micro-cauterization of the MOPPMs causes a marked reduction in the number of scales on the head (A) as compared to the normal head (B).

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be elongated, smaller in size, and divided into two parts by a transverse sulcus. In some instances (45%) the compound eyes were lighter in color — assuming a more coppery tone with irregular streaks of dark brown, in contrast to the more uniform pigmentation of the normal eye.

CONCLUSIONS

From an analysis of the data obtained from these introductory experiments, it would appear that the PPMs of the pupa exercise control over the formation, form and pigmentation of the scales. Further, each pair of PPMs seem to govern a certain area of the body with respect to the scales and not to other external structures — as demonstrated by the control experiment in which wing distortion took place but with no effect upon the scales.

It would appear that the LUPPMs govern scales on the apical portions of the forewings; the LNPPMs govern the scales located on the central plane of the forewings; and the MOPPMs govern the scales on the area of the fronto-clypeus.

As was previously pointed out, treatment by fulguration does not always produce the same results. We believe that this variation is due to the distance between the fulgurating point and the maculae not being constant; thus, the closer the fulgurating point is to the macula the greater the degree of PPM tissue destruction. There is also the difference in age of the pupa together with the difference of one or two seconds when taking time from a stop clock. The addition of all such variable parameters might be responsible for the variation in the effect of cauterization.

Although our experiments are by no means complete, since we are now investigating the other PPMs as well as designing an apparatus that will eliminate the variables mentioned above, we present this paper more in the nature of an "introductory report" with the hope that some of our colleagues interested in the physiology of the Lepidoptera may wish to carry out similar experiments with other species that possess PPMs in the pupal stage, as in the case of many species of Nymphalidae.

Reprints of our previous publication dealing with the method of microcauterization by fulguration will be sent to those of our colleagues interested in work of this nature.

ACKNOWLEDGMENT

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plexippus was made possible by grants to the University of Toronto from the National Geographic Society and the National Research Council of Canada.

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AUTHOR'S NOTE:

Since submitting this paper for publication, we have found that the degree of cauterization must be changed for the various PPMs depending upon the depth of cuticle—thus, a higher degree of cauterization is necessary for the LU as compared to the Al (alar).

By utilizing the scale on the micromanipulator, it is possible to obtain a constant distance between the PPM and fulgurating point.